REMARKS

A. Status of the Claims

Claims 1, 4-13, 15-16, 18-37 and 39-66 are pending in this application with claims 10, 29-36, 41-58 and 62-65 withdrawn from consideration. Claims 1, 4-9, 11-13, 15-16, 18-28, 37, 39-40, 59-61 and 66 have been rejected by the Examiner.

Claims 1 and 16 and dependent claims 4-9, 11-13, 15, 18-28, 37, 39, 40, 59-61 and 66 are rejected under 35 U.S.C. § 112, first paragraph, for allegedly containing new matter.

Claims 25 and 40 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly not enabled.

Claims 1, 4-9, 11-13, 15, 16, 18-28, 37, 39, 40, 59-61 and 66 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite.

Claims 1, 4, 6-9, 11-13, 16, 18-21, 61 and 66 are rejected under 35 U.S.C. § 102(b), for allegedly being anticipated by Pon RA. ("The Study of Polysialic Acid Conjugates," Master's Thesis, University of Ottawa, pp. 1-251, UMI Dissertation Services, 1992, hereafter "Pon") as evidenced by Kabat et al. (J. Exp. Med. 164:642-654, 1986, hereinafter "Kabat").

Claims 1, 4, 6-9, 11-13, 22-24, 26-28, 37, 39, 61 and 66 are rejected under 35 U.S.C. § 102(b), for allegedly being anticipated by Jennings et al. (WO 96/40239, hereinafter "Jennings")

Claims 59 and 60 are rejected under 35 U.S.C. § 103(a), for allegedly being unpatenable over Pon.

B. Explanation of the Amendments

Claims 1, 4-7, 15-16, 37, and 39 have been amended to remove the term "derived" and introduce the term "isolated". Support for these amendments may be found, at least on page 7, lines 11-14 of the specification.

Claims 4 and 39 have been amended to be consistent with the italicized term "*Streptococcus*" as used in claim 5.

Claim 16 has been amended to recite "at least 50%". Support for this amendment may be found, at least, on page 6, lines 12-13 of the specification.

Claim 59 has been amended to recite "at least 95%". Support for this amendment may be found, at least, on page 9, lines 6-7 of the specification.

Claim 37 has been amended to recite "wherein said vaccine generates antibodies that are reactive against the bacteria, yeast or cancer cell from which the polysaccharide or the oligosaccharide was isolated to confer immunity to said cell." Support for this amendment may be found, at least, on page 12, lines 1-6 and 14-17.

Claims 1 and 16 have been amended to recite "or non-naturally occurring protein". Support for this amendment may be found, at least, on page 9, lines 25-27.

Claims 25 and 40 are newly cancelled in this response.

No new matter has been added.

C. The Claims Do Not Contain New Matter

Claims 1 and 16 and dependent claims 4-9, 11-13, 15, 18-28, 37, 39, 40, 59-61 and 66 are rejected under 35 U.S.C. § 112, first paragraph, for allegedly containing new matter. In particular, the Examiner states that the specification lacks support for the limitation "at least

one N-acryloyl group" of the polysaccharide or the oligosaccharide and for the replacement of "at least one removed N-acetyl group with at least one N-acryloyl group". [Office Action dated 4/13/2009, at p. 5]. Applicants respectfully traverse this ground of rejection. Page 8, lines 27-29 of the specification provide the necessary support for this language. The passage reads:

> After hydrolysis, the polysaccharide or oligosaccharide is N-acryloylated to the extent desired by using a variety of acryloylating agents. *(emphasis added)*

The phrase "to the extent desired" indicates that at least one N-acryloyl group, but as many as the user desires, may be added to the polysaccharide or oligosaccharide.

Further support for the recitation "at least one acryloyl group" is provided by the specification at page 9, lines 30-32 that recites "...wherein the protein is linked to the polysaccharide or oligosaccharide through one or more sties on the polysaccharide or oligosaccharide." Since linking would occur through the acryloyl group, this recitation is supported and Applicants respectfully request withdrawal of this ground of rejection.

The Examiner also states that the specification lacks support for the limitation "a polysaccharide or an oligosaccharide", but that support exists for an isolated polysaccharide or oligosaccharide [Office Action dated 4/13/2009, at p. 6]. Solely in the interest of promoting prosecution, Applicants have amended claims 1 and 16 to recite an isolated polysaccharide or an isolated oligosaccharide. Applicants respectfully request withdrawal of this ground of rejection.

D. The Claims Are Enabled

Claims 25 and 40 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly not enabled. In particular, the Examiner alleges a "lack of showing that a de-N-acetylated or an N-acryloylated *Streptococcus* Group B polysaccharide- or oligosaccharide-tetanus toxoid conjugate can be combined with a second monovalent or multivalent protein component, such as, DTaP, or DTP, Td, DTap-Hib, DTaP-IPV-Hib, or combinations thereof, wherein the conjugate still remains optimally 'immunogenic' and produces antibodies reactive against *Streptococcus* Group B., *E. coli* K1, or meningococci." [Office Action, pages 7-8]. Applicants traverse this ground of rejection. Although it is acknowledged that the use of combination vaccines may involve optimizing features of the claimed conjugates and the method of their administration, such optimization which might, for example include substitution of the carrier protein, are within the skill on the art coupled with the teachings provided by Applicants in the description of their invention. Moreover, the art clearly recognizes the advantages and in certain cases need to use multivalent vaccines and it would therefore be inappropriate to deny Applicants' the right to claim their invention as broadly as it is likely to be applied.

The uses of combination vaccines has become a critical component of providing adequate vaccination protocols to very young children. For example, "[u]sing combination vaccines in the routine childhood programme in the United Kingdom amounts to giving 11 injections (24 in the United States), whereas if given separately, 27 (almost 70), would be needed." See, Elliman, D., "Safety and efficacy of combination vaccines", BMJ, 326:995-996 (2003), copy enclosed. Although Elliman recognizes that certain difficulties may arise developing combination conjugate vaccines, Elliman also states that some of these issues might not even be recognized except through good post marketing surveillance of the product. For a

vaccine to even get into a period of market surveillance would imply that during its development and initial marketing those skilled in the art would have had at least a reasonable basis to believe the vaccine would be efficacious. Furthermore, because vaccination is believed to be so critical, simultaneous administration of vaccines is recommended even without all of possible preapproval studies for all of the different possible combinations:

> Preapproval studies of new vaccines commonly include data confirming safety and immunogenicity when the new vaccine is administered simultaneously with licensed vaccines that would be given on a similar or overlapping schedule (3). However, it is impractical to conduct preapproval studies of all combinations that are used in clinical practice. The American Academy of Pediatrics Committee on Infectious Disease states that, "Because simultaneous administration of common vaccines is not known to affect the efficacy or safety of any of the routinely recommended childhood vaccines, if return of a vaccine recipient for further administration is doubtful, simultaneous administration of all vaccines (DTaP [or DTP], OPV or IPV, MMR, rubella, varicella, hepatitis B and Hib vaccines) appropriate for age and previous status of the recipient is recommended. Simultaneous administration of multiple vaccines can raise immunization rates significantly." Guess, H, "Combination Vaccines: Issues in Evaluation of Effectiveness and Safety", Epidemiological Reviews, 21:89-95 (1999), copy enclosed.

Again, the strong endorsement to use simultaneous administration of vaccines supports Applicants' claims to multivalent vaccines. Having provided those in the art with an efficient means of making conjugate vaccines, Applicants' are entitled to claim the various ways in which their vaccines may be used. Accordingly, Applicants request withdrawal of this ground of rejection.

E. The Claims Are Definite Under 35 U.S.C. § 112, ¶ 2

Claims 1, 4-9, 11-13, 15, 16, 18-28, 37, 39, 40, 59-61 and 66 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite.

(a) The Office Action rejects claims 1, 4-7, 15, 16, 37 and 39 for being vague and indefinite for the limitation "derived." In view of the claim amendments to substitute "isolated" for "derived" and the remarks presented above, Applicants believe this rejection is moot. Accordingly, Applicants respectfully request reconsideration and withdrawal of this ground of rejection.

(b) The Office Action rejects claim 1 for allegedly being indefinite, confusing and for lacking antecedent basis for the term: "the polysaccharide or the oligosaccharide is covalently attached to the protein" (lines 9-10). The Examiner states it is unclear where the limitation derives its antecedence from. Although Applicants believe that the following recitation in the claim provides the necessary clarity, "via a β-propionamido linkage between the at least one lysine residue or the at least one cysteine residue and the at least one N-acryloyl group of the polysaccharide or the oligosaccharide" (emphasis added), Applicants have amended the claim as suggested by the Examiner to include that the polysaccharides and oligosaccharides have been N-deacetylated and N-acryloylated. This statement clearly notes that the protein is bound to the N-acryloyl group of the modified saccharides. Accordingly, Applicants respectfully request reconsideration and withdrawal of this ground of rejection.

(c) The Office Action rejects claim 4 for being inconsistent with claim 5 in the non-italicized limitation "*Streptococcus*". Applicants respectfully assert that in light of the claim amendments and remarks presented above, this rejection is moot. Accordingly, Applicants respectfully request reconsideration and withdrawal of this ground of rejection.

(d) Applicants understand claim 39 to be rejected for the same reason as claim 4. Applicants respectfully assert that in light of the claim amendments and remarks presented above, this rejection is moot. Accordingly, Applicants respectfully request reconsideration and withdrawal of this ground of rejection.

(e) The Office Action rejects claim 16 for allegedly being vague and indefinite in the limitation: "about 50%" (line 3). Applicants respectfully assert that in light of the claim amendment deleting the recitation of "about" and remarks presented above, this rejection is moot. Accordingly, Applicants respectfully request reconsideration and withdrawal of this ground of rejection.

(f) The Office Action rejects claim 59 for allegedly being vague and indefinite in the limitation: "about 95%". Applicants respectfully assert that in light of the claim amendments deleting the recitation "about" and remarks presented above, this rejection is moot. Accordingly, Applicants respectfully request reconsideration and withdrawal of this ground of rejection.

(g) The Office Action rejects claim 37 for the following claim language, which is allegedly indefinite and confusing in the limitation: "antibodies reactive against the bacteria...." Applicants respectfully assert that in light of the claim amendments and remarks presented above, this rejection is moot. Accordingly, Applicants respectfully request reconsideration and withdrawal of this ground of rejection.

(h) The Office Action rejects claims 4-9, 11-13, 15, 18-28, 37, 39-40, 59-61 and 66 for allegedly being indefinite because of their dependence on a rejected base claim. Applicants respectfully assert that in light of the claim amendments and remarks above, this

rejection is moot. Accordingly, Applicants respectfully request reconsideration and withdrawal of this ground of rejection.

F. Rejections under 35 U.S.C. §102 (b)

Applicants respectfully traverse the rejection of claims 1, 4, 6-9, 11-13, 16, 18-21, 61 and 66 under 35 U.S.C. § 102(b), for allegedly being anticipated by Pon as evidenced by Kabat. Briefly, the prior art references do not disclose all of the features of the claimed invention. Accordingly, the rejection should be withdrawn.

The Office Action asserts that Pon as evidenced by Kabat allegedly teaches polysaccharide or oligosaccharide-protein conjugates produced via Michael-type addition for use as a vaccine effective against *Escherichia coli* K1 and serogroup B *Neisseria meningitides*. [Office Action, page 11]. Applicants respectfully traverse this ground of rejection. Pon does not disclose Applicants' conjugate vaccines as presently claimed by Applicants. For example, the conjugate referred to in section 4.2.3.3 is made using either BSA or porcine IgG as the protein carrier. Neither of these proteins are either "bacterial or non-naturally occurring" as stated in Applicants' claims. In addition, the conjugate referred to in Table 4-5 is not made by de-N-acetylating and substituting with N-acryloyl, but rather adding an acryloyl group to a terminal NH_2 group added to the reducing end of the colominic acid. See, 4-43 page 183, 4-15 (page 175), and 4-10 (page 172). Thus, this conjugate is fundamentally different from Applicants' claimed conjugates. The sections of Pon the Examiner refers to (tables 5-2, 5-3 and page 205) describe Pon's use of modified saccharides and not the saccharide-protein conjugate molecules of the instant invention. Pon tested modified saccharides alone for antigenicity and the results of these experiments are described in the passages cited by the Examiner. In view of

Applicants' claim amendments, Pon does not disclose Applicants' oligosaccharide-protein or polysaccharide-protein conjugates or their use as vaccines and thus request withdrawal of this rejection.

Applicants respectfully traverse the rejection of claims 1, 4, 6-9, 11-13, 22-24, 26-28, 37, 39, 61 and 66 under 35 U.S.C. § 102(b), for allegedly being anticipated by Jennings. Briefly, Jennings does not disclose all of the features of the claimed invention. Accordingly, the rejection should be withdrawn.

The Office Action asserts that Jennings discloses an N-acyl modified group B meningococcal sialic acid-containing polysaccharide or fragments thereof that is conjugated to a protein carrier. This reference refers to unsaturated group B N-acyl derivative polysaccharides of *N. meningitides* in which the N-acetyl (C_2) groups are replaced by an unsaturated C_{3-4} acyl group as well as protein conjugate vaccines having unsaturated C_{2-4} N-acyl derivatives. The derivatized polysaccharides of Jennings with their unsaturated acyl groups are stated to result in particularly immunogenic conjugates (Jennings, page 4). Unlike the present application, nothing in Jennings discloses or suggests using the unsaturated N-acyl group to form a linkage with protein to make a conjugate via Michael-type addition. In fact, Jennings states that a preferred method of conjugation introduces aldehyde groups in the terminal portion of the polysaccharide through oxidation of vicinal hydroxyl groups and couples the aldehyde groups to the protein amino groups by reductive amination as described in U.S. patent 4,356,170 (Jennings, page 8). In the present application, the presence of the unsaturated N-acyl group is not used for immunogenicity, but to participate in the conjugation reaction with protein so as to result in a saturated β-propionamido-linked polysaccharide-protein conjugate. As Jennings does not teach

all the limitations of the Applicant's claims, the Applicants respectfully request withdrawal of the rejection.

G. Rejections under 35 U.S.C. §103 (a)

Applicants respectfully traverse the rejection of claims 59 and 60 under 35 U.S.C. § 103(a) for allegedly being unpatentable over Pon. Briefly, the prior art reference teaches away from the instant invention.

According to the Office Action, the teachings of Pon do not disclose that the polysaccharide or the oligosaccharide in their conjugates is at least about or at least 95% N-acryloylated. However, optimization of the degree of N-acryloylation of a de-N-acetylated polysaccharide or oligosaccharide was well within the realm of routine experimentation. [Office Action, page 14].

Applicants traverse this ground of rejection because Pon teaches away from producing an immunogenic compound wherein the N-deacetylated polysaccharides or oligosaccharides are at least 95% N-acryloyated as evidenced by pages 202, 209 and table 5-2 of the Pon reference. Pon states,

> It was quite evident that antigenicity of the polysaccharide was lost after 55% of the polysaccharide was de-N-acetylated. *(page 202 of Pon)*

Pon teaches away from modification at the site the Applicants use in the conjugation reaction as it appears to dramatically reduce the antigenicity of the polysaccharide. One of ordinary skill in the art reading Pon would not expect that efficient N-deacetylation at this site (greater than 95% in examples of the instant invention, see page 17, lines 31-32 and page 19, lines 20-21) would result in the levels of antigenicity achieved in the instant invention (see tables 5-8 in the specification) upon N-acryloylation and conjugation. One would, in fact, avoid comprehensive modification at this site based on the teaching of Pon. The Applicants have

shown, unexpectedly based on the teaching of Pon, that extensive modification at this site produces a highly antigenic molecule.

Furthermore, Pon teaches away from the use of bulky or extended additions to the site the Applicants use for conjugation. Page 210 in Pon states,

> There also appears to be a size limitation with the antibody combining site since it is clear that as the substituents become bulkier or more extended, antigenicity falls off.

One of ordinary skill in the art reading Pon would not expect bulky additions like the proteins used in the conjugation reaction presented here to produce immunogenic compounds. However, the Applicants have shown that their novel conjugates are indeed antigenic (see tables 5-8 in the specification). This result is unexpected based on the teachings of Pon.

The Applicants respectfully request the withdrawal of the rejection.

CONCLUSION

Based on the foregoing amendments and remarks, Applicants respectfully request reconsideration and withdrawal of the rejection of claims and allowance of this application.

AUTHORIZATION

The Commissioner is hereby authorized to charge any additional fees which may be required for consideration of this Amendment to Deposit Account No. **50-3732**, Order No. **13564-105037US2**.

Respectfully submitted,
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Epidemiological Reviews
Vol. 21, No. 1

Printed in U.S.A.

Combination Vaccines: Issues in Evaluation of Effectiveness and Safety

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INTRODUCTION

Combination vaccines, which help provide protection against two or more diseases or against multiple serotypes of a single disease are increasingly used to help reduce the number of injections required for childhood immunization (1). Familiar combinations include measles, mumps, and rubella (MMR); diphtheria, tetanus, and whole-cell pertussis (DTP); poliomyelitis (serotypes 1–3); pneumococcal disease (23 serotypes); and influenza A and B. Recently a number of diptheria, tetanus, and acellular pertussis (DTaP) vaccines have received licensing approval, and DTaP is now recommended as the preferred combination in the United States, with DTP vaccine as an acceptable alternative (2). In the United States there have been only two recently licensed vaccines combining moieties that had previously required separate injections. A combination vaccine for *Haemophilus influenzae* type b (Hib) and hepatitis B (COMVAX®, Merck & Co., Inc., Whitehouse Station, NJ) is available for use beginning at 2 months of age and a combination DTaP/Hib vaccine (TriHIBit®, Pasteur Mérieux Connaught, USA, Swiftwater, PA) is available for use at 15 months of age. Further combinations involving DTaP, Hib, inactivated poliomyelitis vaccine (IPV), and hepatitis B are licensed in Canada and European countries, and additional combinations, including MMR and varicella, are in clinical trials.

Regulatory guidance on formulation, manufacturing, preclinical testing, and pre-licensure clinical trials of combination vaccines has recently been provided by the US Food and Drug Administration (FDA) (3). While the immunologic, manufacturing, and regulatory aspects of combination vaccines have been the subject of a number of reviews, less has been written on the evaluation of effectiveness and safety. In what follows, we will review the recent literature on the epidemiologic evaluation of effectiveness, safety, and potential improvements in vaccine acceptance and coverage associated with combination vaccines.

SEROLOGIC RESPONSES AND THEIR RELATION TO CLINICAL EFFICACY OF COMBINATION VACCINES

There are a number of physical, chemical, and immunologic mechanisms by which serologic responses to antigens in combination vaccines may differ from those obtained with separate administration of the components (3, 4). Preservatives used with one component may alter the potency of other components. For example, thimerosal, a preservative in DTP vaccines, has long been known to adversely affect the potency of IPVs (5, 6). Buffers used with different components may be incompatible (4). Antibody titers to some live viruses may be lower when administered in a combination vaccine than when administered separately (7). Reduced antibody responses have also been shown when multiple protein-conjugated vaccines sharing common protein epitopes have been administered simultaneously (8). Some combination vaccines and vaccine mixtures containing Hib antigens have been shown to have a lower Hib antibody response than has been seen with separate administration, although the clinical implications of the lower responses have not been established (9–12).

Serologic correlates of efficacy have been established for a number of diseases (3, 13). Several lines of indirect evidence indicate that serum neutralizing antibodies to poliovirus induced by natural infection, oral poliomyelitis vaccine (OPV) or IPV are protective against paralytic disease (5). Antibodies to the hepatitis B surface antigen induced by vaccination or by natural disease have been correlated with protection on the basis of animal challenge experiments and vaccine clinical trials in humans (14, 15). Serum bacteriocidal activity against *Neisseria meningitidis*, serum anti-

Received for publication January 5, 1999, and accepted for publication March 25, 1999.

Abbreviations: CDC, Centers for Disease Control and Protection; DTaP, diphtheria, tetanus, and acellular pertussis; DTP, diphtheria, tetanus, and whole-cell pertussis; FDA, US Food and Drug Administration; GMT, geometric mean titers; Hib, *Haemophilus influenzae* type b; HMO, health maintenance organization; IPV, inactivated poliovirus vaccine; MMR, measles, mumps, rubella; OPV, oral poliomyelitis vaccine; PRP, polyribosyl ribitol phosphate; VAERS, Vaccine Adverse Events Reporting System.

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toxin activity against diphtheria and tetanus, antibody to the hepatitis B surface antigen, and antibodies to serotype-specific capsular polysaccharides of *Streptococcus pneumoniae* have all been shown to be correlated with protection (13–16). Antibodies to the Hib capsular polysaccharide (polyribosyl ribitol phosphate (PRP)) have also been correlated with protection (17).

However, as noted by the FDA (3) and by Granoff and Lucas (17), the quality and not simply the quantity of the antibody also needs to be considered in comparative immunogenicity studies of combination vaccines. For example, antibodies with a low functional affinity for the Hib-PRP show lower percent binding at low antigen concentrations (a measure of low avidity). This suggests that the extent to which a given level of vaccine-induced antibody to PRP protects against clinical Hib disease may be influenced by the avidity of the antibody (17). In addition, recent experience with acellular pertussis vaccines shown to be effective in protecting against clinical disease has indicated that levels of antibody induced by vaccines are not predictive of protection (13, 18). Several markers of cell-mediated immunity to pertussis antigens may ultimately prove more useful than serology in predicting vaccine-induced protection against pertussis (13, 19).

Thus, factors other than antibody levels or seroconversion rates (percentage of vaccinees achieving a previously established "protective" level of antibody) need to be considered in the immunologic evaluation of combination vaccines (3). For example, markers of immunologic memory have proven to be important for evaluating the potential for long-term protection of some vaccines (20–22). One way in which evidence of immunologic memory can be provided is through demonstrating production of an anamnestic immunoglobulin G antibody response to revaccination or exposure to natural disease (23). Antibody avidity assays have also been used as surrogate markers of immunologic memory (24). Hence, even if there are well-established protective antibody levels applicable to the individual antigens comprising a new combination vaccine, it would be important to provide evidence that the previously established protective levels can be validly applied to the new combination vaccine.

PREAPPROVAL SAFETY AND EFFICACY EVALUATION OF COMBINATION VACCINES

Formulation and testing requirements

Prior to any human clinical evaluation of new components of combination vaccines, extensive preclinical testing is required to ensure compatibility and stability of the proposed new formulations, as well as consistency of the final manufacturing process (25). When there are suitable animal models for the diseases, both the individual components and their combination should be tested for animal immunogenicity and for protective efficacy in animal challenge studies (3). Safety testing in animals will also generally be required for any component for which such data are not already available. If adjuvants are used that have not been used previously in currently licensed vaccines, animal toxicology testing of the adjuvants may be required (3). The preclinical development of any combination vaccine is typically carried out as an iterative process, as formulations are modified and refined on the basis of in vitro and in vivo assays.

Preapproval testing of combination vaccines for tolerability and immunogenicity in man

Each new component will generally be initially evaluated individually for immunogenicity and tolerability in adults, unless the new component has already undergone human clinical testing in the formulation to be used in the new combined vaccine. Once this initial testing has shown a new component to be immunogenic and well tolerated, it will then typically be evaluated in a clinical immunogenicity trial where it is administered separately but simultaneously with the existing vaccine. All of this should be done before the new component is tested as part of a combined vaccine. It is important to recognize that from a regulatory perspective, a combination vaccine is a new vaccine, which must undergo regulatory review and licensing approval, even if all of the moieties are from approved single-component vaccines (3).

On purely statistical grounds one could argue that 2^N arms would be required in a clinical trial to evaluate immunogenicity of an N-component combination vaccine if there was no biologic basis for eliminating any of the theoretically possible interactions from consideration (26). In practice, it is rarely necessary to test all possible combinations of components. Most comparative clinical immunogenicity trials of combined vaccines are conducted with previously approved combinations and/or separate components as active controls. Nonetheless, the statistical multiplicity in comparing immune responses to large numbers of different components can lead to very large sample sizes and can sometimes make apparent differences in immunogenicity of individual components difficult to interpret.

Evolving standards for the conduct of equivalence trials provide a paradigm for vaccine studies to establish serologic equivalence between a given combination vaccine and the individual component antigens of which it is composed (27, 28). A common problem is

to establish that the seroconversion rate for a vaccine component administered as a combination (R_c) is not lower by a predefined clinically important amount, Δ, than that obtained when the component is administered separately (R_s). Typically, the magnitude of the amount, Δ, will have been agreed upon in discussions with the FDA before the trial is undertaken.

As outlined by Blackwelder (27), the statistical test to establish non-inferiority may be formulated by taking the null hypothesis to be H_0: $R_c \leq R_s - \Delta$. Rejection of H_0 at a significance level of α is required to conclude that $R_c > R_s - \Delta$. Hypothesis tests regarding comparisons of geometric mean titers (GMTs) would be formulated similarly, except that it may be more appropriate to test for equivalence rather than non-inferiority, with an obvious modification of the above equations. If it were necessary to compare other immunologic parameters such as avidity or markers of cell-mediated immunity, the same type of statistical considerations would apply. Because all of these comparisons would typically need to be established simultaneously for each component of a combination vaccine, the required sample sizes can be quite substantial.

Throughout the entire process of preapproval clinical trials, there will be extensive monitoring for local reactions, systemic complaints, and serious adverse events. Even if all of the components of the combination are themselves licensed vaccines, it is still necessary to evaluate the safety and tolerability profiles of the combination.

Evidence of clinical efficacy required for approval of combination vaccines

If immunologic correlates of efficacy are well established for each component of a combination vaccine, data from immunogenicity trials could provide a basis for license approval without the need for additional evidence studies (3). When immunologic data are not sufficient, the type of evidence needed for approval will depend on the specific combination vaccine under consideration. For example, recent approvals of combination vaccines containing diphtheria, tetanus, and acellular pertussis antigens required randomized, double-blind, clinical efficacy trials to demonstrate that the new pertussis components were protective against clinical disease. However, immunologic data were relied upon to show that efficacy of the diphtheria and tetanus antigens had been maintained.

Clinical efficacy trials of combination vaccines for multiple serotypes may have as a primary endpoint the aggregate of disease with all serotypes included. However, the study should be sufficiently powered to permit meaningful subgroup analysis of protection against some individual serotypes (3). It is important to consider differences between the serotype distribution in the population in which efficacy trials are conducted and those in which the vaccine will subsequently be used. Such differences can lead to important differences between the efficacy shown in clinical trials and that found with use of the vaccine in clinical practice after licensure.

EPIDEMIOLOGIC STUDIES OF COMBINATION VACCINE EFFECTIVENESS IN CLINICAL PRACTICE

Efficacy and effectiveness

Once a vaccine has been licensed on the basis of preclinical studies and clinical trials, demonstrating its safety and efficacy, it is important to evaluate its effectiveness in clinical practice (29, 30). *Efficacy* of a vaccine refers to the reduction in disease measured in a carefully-monitored, randomized controlled clinical trial conducted in a homogeneous population according to a defined protocol. *Effectiveness* refers to the reduction in disease measured under conditions of use of the vaccine in ordinary clinical practice (31). For most pharmaceutical interventions, effectiveness would be expected to be somewhat less than efficacy. The original polysaccharide Hib vaccines may have been an example of a type of vaccine whose effectiveness was less than its efficacy.

On the other hand, Hib conjugate vaccines represent at least one type of intervention whose field effectiveness in elimination of invasive Hib disease may actually be greater than what would have been predicted from immunization rates and efficacy in clinical trials (32). It appears that widespread vaccine use decreased the transmission of the disease to non-vaccinees, thereby causing a decreased likelihood of disease among both vaccinees and non-vaccinees. A possible mechanism is through lower nasopharyngeal carriage of the organism in vaccinated children than in unvaccinated children (33). This example of a vaccine whose effectiveness may be greater than its efficacy provides a further illustration of the importance of monitoring vaccine effectiveness after licensure, as emphasized by Orenstein et al. (34).

Commonly used designs for observational studies of vaccine effectiveness

Epidemiologic studies of vaccine effectiveness in clinical practice generally involve either direct (cohort) or indirect (case-control) comparison of the incidence of disease in vaccinated and non-vaccinated individuals using standard methods that have been well described in a number of reviews (29, 34). A

design more commonly used in health policy research than in epidemiology is the "interrupted time series" (35). This has been used to estimate the effect of policy changes on health outcomes, while controlling for temporal trends (36). A similar method has recently been used to estimate the effects of interrupted pertussis vaccination programs on disease rates by comparing incidences in countries which have maintained high pertussis immunization rates with incidences in countries in which program interruptions have occurred (37).

Variation in effectiveness with time since vaccination or season of vaccination has been studied not only with traditional cohort designs but also with case-control designs (38, 39) and, more recently, with a nonparametric design (40). More complex mathematical modeling has been used to evaluate potential long-term epidemiologic effects of new immunization policies (41, 42) and to predict disease outbreaks in partially immunized populations (43).

Because the efficacy of most vaccines is typically at least 50 percent, effects of selection bias, unmeasured confounding, misclassification, and other non-random sources of error are typically capable of being overcome by careful study design. For example, a randomized, double-blind, placebo-controlled clinical trial of influenza vaccination in healthy elderly patients in Holland (44) and a retrospective cohort study of influenza vaccine effectiveness among residents of a Minnesota health maintenance organization (HMO) (45) produced very similar measures of overall vaccine effectiveness. The randomized trial used a more rigorous study design and a more stringent case definition. However, the observational study was able to evaluate not only more clinically important endpoints, including hospitalization and mortality, but also was capable of quantifying the cost-effectiveness of the intervention. These two studies, which were undertaken quite independently, illustrate how observational studies and randomized clinical trials can be used together to provide additional information about vaccine effectiveness.

Potential problems to be faced in comparative observational effectiveness studies

Observational studies to quantify differences in effectiveness of two vaccines are typically much more sensitive to bias than observational studies of the absolute effectiveness of a vaccine, because the effect size is typically larger in the latter type of study. Consequently, it is difficult to use observational studies to determine whether differences in surrogate markers of efficacy between two licensed combination vaccines actually translate into differences in effectiveness. For example, study design differences have made it difficult to interpret conclusions from observational effectiveness studies of several acellular pertussis vaccines (46). As was discussed in the previous section, it is difficult enough to conduct an equivalence trial of a multicomponent vaccine using a randomized design, where the main problem lies in the large sample sizes required. Observational designs are typically only useful when comparing vaccines with very large differences in effectiveness. For example, it may be possible to use observational studies to demonstrate an advantage of one vaccine over another in providing greater protection after the first dose of a series. Large simple randomized clinical trials continue to be by far the preferred approach for providing valid estimates of moderate differences in effectiveness between two efficacious vaccines.

EPIDEMIOLOGIC RESOURCES FOR SAFETY STUDIES OF COMBINATION VACCINES

The National Childhood Vaccine Injury Act of 1986 requires health care providers who administer vaccines in the United States to maintain permanent vaccination records and to report occurrences of certain adverse events to the Vaccine Adverse Events Reporting System (VAERS) (47). The annual number of reports now exceeds 10,000 per year (48). While this system of passive reporting provides a basis for identifying potential vaccine-related adverse events, it is not well suited to analytical studies. Despite the requirement for reporting, there is a substantial degree of underreporting in VAERS compared with reporting in controlled studies (49).

Rates of underreporting in VAERS also vary according to a number of unknown factors, creating the potential for bias in comparisons of vaccine safety based on passive reporting in VAERS. To evaluate this phenomenon, a retrospective cohort study was performed to compare rates of adverse events in children who had received one of two recombinant hepatitis B vaccines (50).

In VAERS, rates of serious adverse events were approximately three times higher with one vaccine than the other ($p < 0.01$). A serious adverse event was defined to be one that resulted in hospitalization, permanent disability, or death, or was judged to be life threatening. The cohort study was conducted with the Centers for Disease Control and Protection (CDC) Vaccine Safety Datalink Project, which makes use of linked medical records and vaccine records on more than 500,000 children aged 0–6 years enrolled at four large HMOs in the western United States (48). In contrast to the pronounced difference in reporting rates seen in VAERS, the rates of all hospitalizations within 30 days after receiving either of the two vaccines were

nearly equal (relative risk = 1.04, 95 percent confidence interval: 0.93, 1.15). An additional analysis addressed hospitalizations or emergency room visits for diagnostic categories that represented either events commonly reported to VAERS after hepatitis B vaccination in infants or else preselected by the investigators as being possibly vaccine related. In this analysis the rates were again essentially equal (relative risk = 1.07, 95 percent confidence interval: 0.94, 1.22). This study underscores the importance of undertaking analytic epidemiologic studies to evaluate findings from passive surveillance systems.

The Vaccine Safety Datalink Project was funded by CDC in 1991 and represents the largest comprehensive database resource for analytic studies of vaccine safety in the United States (48). Initially the project focused on children aged 0–6 years but it has been expanded to include adolescents and adults. Health service use information for each subject is computerized on an individual patient basis and organized into files containing demographics and enrollment, vaccine administration records (including date, manufacturer, lot number, vaccination site, and whether the vaccinations were obtained within the HMO or from outside providers), hospital and emergency room visits (at all four sites) and outpatient visits (at two sites), selected diagnostic procedures and laboratory data, prescription drugs, linkage to state birth and death certificates, geocoding (for estimation of socioeconomic status based on census block codes), and past medical diagnoses. Written medical records are available for review. Numerous data quality control procedures have been instituted to ensure accuracy and completeness of the information and to monitor and maintain quality of the records abstraction process. Software has been developed to permit computing person-time at risk in various defined time windows after vaccination. A number of specific analytic methodologies have been employed, depending on the specific question to be addressed.

Safety studies of combination vaccines published to date have included 1) the risk of chronic arthropathy among women following rubella vaccination (51), 2) rates of serious clinical events in recipients of MMR vaccine at 4–5 years and 10–12 years (52), 3) timing of seizures following DTP vaccination and MMR vaccination (43), and 4) the risk of hospitalization because of aseptic meningitis after MMR vaccination (53).

The VAERS and the CDC Vaccine Safety Datalink Project complement each other. VAERS provides a mechanism for early identification of potential safety problems with new vaccines used singly or in combination with existing vaccines. The Safety Datalink Project provides a mechanism for analytic studies, quantitative risk estimation, and comparative studies. Together they represent a unique national resource for monitoring vaccine safety. In particular, these resources are especially useful for studying the safety of combination vaccines and simultaneous use of multiple vaccines as they are administered in clinical practice.

SIMULTANEOUS ADMINISTRATION OF VACCINES

Preapproval studies of new vaccines commonly include data confirming safety and immunogenicity when the new vaccine is administered simultaneously with licensed vaccines that would be given on a similar or overlapping schedule (3). However, it is impractical to conduct preapproval studies of all combinations that are used in clinical practice. The American Academy of Pediatrics Committee on Infectious Disease states that, "Because simultaneous administration of common vaccines is not known to affect the efficacy or safety of any of the routinely recommended childhood vaccines, if return of a vaccine recipient for further immunization is doubtful, simultaneous administration of all vaccines (DTaP [or DTP], OPV or IPV, MMR, rubella, varicella, hepatitis B, and Hib vaccines) appropriate for age and previous vaccination status of the recipient is recommended. Simultaneous administration of multiple vaccines can raise immunization rates significantly" (54, p. 21). Simultaneous administration of all vaccines for which a child is eligible at the time of the visit is also one of the Standards for Pediatric Immunization Practices recommended by the National Vaccine Advisory Committee and approved by the US Public Health Service (55). Ecologic analyses of reports from passive surveillance systems for monitoring reports of adverse events among vaccinees suggest that simultaneous administration of common pediatric vaccines do not appear to have been associated with increased reports of serious adverse events (56).

The comment that simultaneous administration of multiple vaccines can raise immunization rates is supported by a decision analysis performed in the Northern California Kaiser Permanente Medical Group (57). Analysis of charts of 4,691 children who had missed one or more immunizations due in their second year showed that about one third of underimmunized children in the Northern California Kaiser Permanente Medical Group would have received all their second year immunizations if their providers had followed the guideline to administer simultaneously all vaccine doses for which the child was eligible. Despite this finding and the above recommendations, a national survey of a random sample of pediatricians and family practitioners found that approximately one third would not vaccinate an 18-month-old healthy

child with all four vaccines because of perceived medical contraindications, concerns about pain, parental objections, or costs (58). It appears likely that increased use of combination vaccines can help overcome some of the reluctance of practitioners to administer multiple vaccines simultaneously by reducing the number of injections necessary to deliver childhood vaccinations.

CONCLUSIONS

Combination vaccines are expected to become increasingly common and increasingly complex over the next decade as additional antigens are added to the pediatric vaccination regimen (59, 60). This should permit a further reduction in childhood infectious diseases and lessen the potential dangers of increasing antibiotic resistance among organisms causing serious childhood disease. At the same time it will be necessary to develop methodology to know how to use the vaccines most efficiently and to monitor their safety, effectiveness, and effects on immunization coverage in practice.

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option for China and Africa. In contrast several agricultural strategies reduce the quantity of aflatoxin in food. Genetic modification of crops to enhance fungal resistance is a promising method and biocontrol by flooding fields with non-toxigenic fungi is another. But much of the contamination of food occurs after the harvest and during storage. Methods to reduce humidity can limit fungal growth. Drying the crop in the sun, on a mat, discarding visibly mouldy kernels or nuts before storage, and using natural fibre sacks for storage and placing these on wooden pallets to keep the crop dry can be very effective.[8 10] Rural communities can use these techniques at minimal expense. We urgently need to evaluate their impact on human exposure to aflatoxin and implement them for the benefit of existing hepatitis B carriers—who make up 15-20% of many populations at high risk.

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Competing interests: None declared.

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Safety and efficacy of combination vaccines

Combinations reduce distress and are efficacious and safe

For 130 years or more after Jenner introduced a vaccine for smallpox this was the only vaccine in general use. Ten vaccines are now included in the routine childhood vaccination programme in the United Kingdom , with multiple doses of most. The use of combination vaccines reduces distress to the recipients and is likely to increase uptake rates. Many combinations are as efficacious as the separate vaccines, but the increasing number of antigens could theoretically pose problems in terms of reduced immunogenicity or increased reactogenicity.

Good post-marketing surveillance will become important in monitoring both the clinical efficacy of combination vaccines and adverse effects. With respect to clinical efficacy this may be a particular problem with combination conjugate vaccines. Using combination vaccines in the routine childhood programme in the United Kingdom amounts to giving 11 injections (24 in the United States), whereas, if given separately, 27 (almost 70 in the United States) would be needed. The alternative approaches are combining as many antigens into as few injections as possible, giving multiple simultaneous injections, or giving the required vaccines over several visits. Generally parents tend to have fewer concerns than health professionals about multiple injections.[1 2] However, it would seem cruel to give more injections than required. In addition, if many injections are due at the same time, some may be delayed or not given at all.[3] Pentavalent vaccines such as diphtheria, tetanus, wholecell pertussis vaccine (DTwP), *Haemophilus influenzae* type B (Hib) vaccine, and inactivated polio vaccine (IPV) are widely available. Hexavalent vaccines such as diphtheria, tetanus, acellular pertussis vaccine (DTaP), hepatitis B virus (HBV) vaccine, IPV, and Hib are being developed.

The safety, efficacy, and immunogenicity of a combined vaccine may be affected by interactions, not only between the antigens but also between these and other components such as adjuvants, stabilisers, and preservatives. Research on combination vaccines is more difficult than on single antigen vaccines because they are often replacing widely used single vaccines, making trials with placebos unethical. The disease may no longer be common, so the production of antibodies or immunogenicity, rather than protection from disease (clinical efficacy), has to be assessed. This may be satisfactory when antibody concentrations correlate closely with protection, but for some diseases (for example, pertussis) this is not the case. Thus post-marketing surveillance is essential.

Combining vaccines into one product does not increase the overall rate of adverse events, and with some combinations, such as DTaP, the rates are lower than when the component vaccines are given separately.[4] Schmitt et al compared antibody responses in children receiving DTaP-HBV-IPV-Hib as one injection with children receiving the same antigens but with the Hib given at a different site. No difference was found in adverse events associated with the different regimens.[5]

In 1998 a paper in the *Lancet* was interpreted as showing a link between measles, mumps, and rubella vaccine and pervasive developmental disorder and bowel disease,[6] even though the authors said they had not proved such a link. Subsequent research has failed to find evidence for this link [7] The suggested mechanism behind the hypothesis was that combining antigens produced an unpredictable response. Some parents are concerned that multiple antigens may overload the infant's immune system. A recent review set in context the antigenic load from vaccines in com-

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parison with that from the environment and emphasised the capacity of the immune system to respond effectively to numerous simultaneous antigens.[8] Using data linkage, Miller et al found no evidence for an increase in admissions to hospital for serious bacterial infections following MMR vaccination.[9]

One disadvantage of giving vaccines in combination is that it may not always be clear which component is responsible for a particular adverse event. As important as safety is ensuring that combining antigens does not compromise the protection afforded by each antigen. In the study by Schmitt et al, no difference was found in subjects achieving protective concentrations of antibodies against diphtheria, tetanus, hepatitis B, and polio.[5] Concentrations of pertussis antibody were the same for both groups and comparable with those achieved in trials of DTaP alone. However, the concentrations of Hib polyribosylribitol phosphate (PRP) antibody were statistically significantly lower in those children receiving all the antigens mixed together. The clinical significance of this is uncertain.

One of the longest established combination vaccines is DTwP. Two Swedish vaccine trials found a significant difference in post-immunisation levels of diphtheria antitoxin depending on the presence and nature of any pertussis antigens in the vaccine.[10] The addition of an efficacious wholecell pertussis (wP) component to diphtheria and tetanus vaccine increased the geometrical mean titre of diphtheria antitoxin in the recipients, whereas the addition of acellular pertussis (aP) or a poorly efficacious wholecell pertussis vaccine produced lower concentrations than only diphtheria and tetanus vaccine. In a few children, the concentrations reached were considered non-protective, confirming the well known adjuvant effect of efficacious wholecell pertussis vaccines. DTwP vaccines can be combined with Hib vaccines with no clinically significant loss in immunogenicity, but when DTaP is used instead lower concentrations of Hib PRP antibodies have been observed,[11] and in some cases these are below protective levels. The clinical significance of this was unclear.

However, there has been a rise in Hib cases in fully immunised children in the United Kingdom. This is probably in part due to the use of a combined DTaP/Hib preparation.[12] Dagan et al reported that infants who were given a diphtheria-tetanus-pertussis-polio-Hib vaccine, in which the Hib component was conjugated to tetanus, simultaneously with a pneumococcal vaccine also conjugated to tetanus toxoid had lower Hib PRP antibody concentrations than infants who had received pneumococcal vaccine conjugated to diphtheria toxoid.[13] Furthermore, children who had received higher doses of pneumococcal tetanus conjugate had poorer responses. This implies that difficulties may arise in using simultaneous or combined vaccines that have conjugates in common.

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Management of bacterial meningitis in adults

Algorithm from the British Infection Society represents current standard of care

The treatment of bacterial meningitis represents one of the success stories of modern medicine, particularly antibiotics. In the pre-antibiotic era bacterial meningitis was almost always fatal, but the prompt use of appropriate antibiotics together with supportive care can undoubtedly reduce the morbidity and the mortality of this condition substantially. And yet just 10 years ago a large study of acute bacterial meningitis in adults found a mortality of 25%.[1] Why can't we do better than that?

Acute bacterial meningitis tends to present to non-specialist, and often inexperienced, junior doctors. It is not very common—there are about 1000 patients in the United Kingdom each year—and so individual doctors will not see many patients. These are exactly the circumstances in which a management algorithm can help. The British Infection Society has recently published such an algorithm for the initial management of adult patients with presumed bacterial meningitis,[2] and which represents an updated version of the evidence based recommendations published by the society four years ago.[3] Key to the success of algorithms such as this one is simplicity. The new guidelines recommend a third generation cephalosporin such as

BMJ 2003;326:996–7